urologist, of course, the ideal arrangement would seem to be that all genito-urinary organs are his sphere, exclusively. But as yet the borderline is not so distinctly delimited. The establishment of this long-desired Board of Urology will be a crucial step forward toward the time when the dabbler in cystoscopy, for instance, who tries to do the work of a specialist, will be outlawed. Likewise, the general surgeon or medical man—untrained in urology and lacking certification by the Urology Board—who attempts the resection of a prostate will not be tolerated.

We have no grievance against the physician or surgeon who feels that he is entitled to use some or all of the armamentarium of the urologist, provided always that he has been trained to do this; provided, also, that his skill shall be continuously, and not occasionally, employed, and that he be familiar with developments taking place in this specialty. But urologists who, as we have seen, have lifted their specialty out of the depths, out of laymen's hands, may confidently expect that ultimately we, as specialists, shall be restricted to the practice of urology, and that—the work of urologically competent physicians and surgeons excepted—that practice shall be definitely limited to us.

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SERUM PRESERVATIVES*

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DISCUSSION *by K. F. Meyer, Ph. D., University of California.*

RECENT demands for the preparation of human serum for parenteral injection have resulted in the use of a variety of technical procedures, probably in no instance meeting the federal standards for serum production. There is indeed, perhaps, no situation in which state and local standards vary so widely from federal standards as is true in the realm of biologic products. Yet each move in the development of federal standards has represented careful thought, careful tests, and a background of experience which is not generally recognized.

PRESERVATIVES IN SERUM AN OLD PROCEDURE

The use of preservatives in serum has been a procedure of long standing. Present practice has made it evident that the background for this step is not understood. In brief, serum produced under federal regulations is usually chemically purified, and, even if not, is filtered. Preservative is added to a product which is supposedly sterile, and the mixture not only stands for long periods before it is released, but repeated tests for sterility, carried for seven days each, animal inoculations for tetanus and for excess of preservative, all are carried through.

SERUM PRESERVATIVES MUST BE USED WITH CARE

It may be granted that emergencies have demanded shorter methods, but one should not lose sight of the fact that serum preservatives have been used with care and with one motive, namely, to prevent spoilage of a very exceptional bottle which, in spite of all tests, might be lightly contaminated. By all proper legal procedures, these contaminations would have to be very rare, and of these rare contaminations, pathogens would form the smallest percentage, In other words, our practice with regard to preservatives has never been intended to take care of anything but accidents, against which every precaution is taken. That it is thus illogical to make the assumption that the addition of preservative offsets other errors becomes evident; yet recent conditions have made it clear that the point is not well understood.

STERILITY TESTS LIKELY TO MISLEAD

Another fundamental point has been missed in the rush of events. The range of activity of preservatives falling within limits which prevent bacterial development, but do not kill, is not clean-cut. Cell functions may be variously upset without destruction of the organism; and, under proper conditions, normal functions may be resumed. In other words—and this should be clearly emphasized—tests for the presence of living organisms in a preserved product are by no means a

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TABLE 1.—*Subcultures from Serum Inoculated with Staph. aureus, Preserved with Merthiolate*

	1:5,000		1:10,000		1:50,000		Control	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
*Immediate	+	....	+	....	++++	.....	++++	.....
20 hours	+	—	+	—	++++	+++	++++	++++
44 hours	—	—	+	—	+++	—	++++	++++
6 days	—	—	+	—	+++	+++	++++	++++

* Since the organisms in serum at this time must have been nearly 100 per cent living, this "immediate" series serves as a bacteriostatic control on technic, and renders the interpretation more cautious, *i. e.,* bacteriostasis in subcultures is marked but not complete, in agar, but is complete in broth.

simple matter of broth or agar inoculation in the usual manner. On one extreme, broth inoculated with preserved serum may show growth, if living organisms are present, in a few hours; on the other extreme, broth so inoculated will never show growth, although living organisms are inoculated. Between the extremes, broth may show growth after a decided delay, which accounts for the seven day federal sterility test. Actual delay probably rarely exceeds seven days, except with spores, but sight should not be lost of the fact that living organisms may not be revealed by this technique at all. This latter extreme is by no means unusual. It is due, of course, to the fact that the preservative carried to broth with the inoculated serum is sufficient to prevent the multiplication which reveals itself as turbidity of the broth. A good preservative is a good inhibiting agent. But it is by no means reasonable to argue that, because the small amount of preservative carried to broth prevents growth in broth, hence the greater concentration of preservative in serum makes that serum foolproof. Organisms can still remain viable in the serum for days, and, on

TABLE 2.—*Subcultures from Serum Inoculated with B. Coli, Preserved with Merthiolate*

	1:5,000		1:10,000		1:50,000		Control	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
*Immediate	+	....	++	....	+++	.....	++++	.....
20 hours	+	—	++	—	++++	++++	++++	++++
44 hours	—	—	+	—	++++	++++	++++	++++
6 days	—	—	+	—	++++	++++	++++	++++

TABLE 3.—*Subcultures from Serum Inoculated with Staph. aureus, Preserved with 1:5,000 Merthiolate, 1:1,500 Chinosol, and held at Room Temperature*

	Merthiolate		Chinosol		Control	
	Agar	Broth	Agar	Broth	Agar	Broth
*Immediate	+	....	++	.....	++++	.....
20 hours	+	—	+	—	++++	++++
44 hours	—	—	++	++++	++++	++++
6 days	+	—	+	++++	++++	++++

TABLE 4.—*Subcultures from Serum Inoculated with Staph. aureus, Preserved with Chinosol*

	1:1,000		1:1,500		1:3,000		Control	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
*Immediate	+	.....	++	.....	++++	.....	++++	.....
20 hours	++	++++	+	++++	+++	++++	++++	++++
44 hours	++	++++	—	+++	++	++++	++++	++++
6 days	++	++++	++	++++	++	++++	++++	++++

TABLE 5.—*Subculture from Serum Inoculated with B. Coli, Preserved with Chinosol*

	1:1,000		1:1,500		1:3,000		Control	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
*Immediate	++++	.....	++++	.....	++++	.....	++++	.....
20 hours	++++	++++	++++	++++	++++	++++	++++	++++
44 hours	+++	++++	++++	++++	++++	++++	++++	++++
6 days	++++	++++	++++	++++	++++	++++	++++	++++

parenteral injection, might logically find conditions suitable for development.

NOTATIONS CONCERNING CERTAIN PRESERVATIVES

General practice has given us, as preservatives for serum, tricresol, chinosol and, more recently, merthiolate. Based on the prevalent misconceptions in the rush of recent events, we have attempted brief tests of tricresol, chinosol, merthiolate, glycerin and formalin. Although some discrepancies have arisen as to the manner of using these products, the general recommendations are:

Tricresol.—Final concentration, 0.2 per cent, using for stock solution a mixture of one part tricresol with one part ether.

Chinosol.—Final concentration 1:1,500, using 3.3 c.c. of a 2 per cent aqueous solution of chinosol per 100 c.c. of serum.

Merthiolate.—Final concentration 1:5,000 (also used 1:10,000), using a 1 per cent aqueous solution of merthiolate crystals. This is usually acid, and the crystals will precipitate in a few days, although not in serum. The addition of 1.4 grams of C. P. borax per 100 c.c. of aqueous 1 per cent merthiolate has been recommended.

Glycerin.—Final concentration 1 per cent.

Formalin.—Final concentration 0.2 per cent, based on the use of straight formalin (usually 35 to 40 per cent formaldehyde) neutralized with NaOH when used.

HOW THESE PRESERVATIVES SHOULD BE USED

Regarding the uses of these products, as to general practice and suitability, the following observations may be pertinent:

TABLE 6.—*Subcultures from Serum Inoculated with Staph. aureus, Preserved with Tricresol, Glycerin, and Formalin*

	Tricresol		Glycerin		Formalin		Control	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
*Immediate	+++	++++	++++	++++	++	++++	++++	++++
48 hours	++++	++++	++++	++++	++++	—	++++	++++
5 days	++	++++	++++	++++	+	—	++++	++++

Tricresol.—Used for years in standard antisera, but ordinarily added to dialysed serum concentrates from which a major fraction of protein has been removed, and which have been sterilized by filtration. It is thus not relied upon as more than an inhibiting agent for contaminants which presumably do not exist, but might theoretically be present in extremely low concentration.

Chinosol.—Has been used for poliomyelitis serum and others for some years, but very little evidence on its effectiveness seems to be available.

TABLE 7.—*Subcultures from Serum Inoculated with an Unknown Coccus (Found in Human Serum), Preserved with Tricresol, Glycerin, Formalin*

	Tricresol	Glycerin	Formalin	Control
*Immediate	+++	++++	++	++++
48 hours	+++	++++	—	++++
5 days	+++	++++	—	++++

Delayed growth, an indication of attenuation and/or reduction in numbers, was apparent frequently, and does not appear from these tables. It appeared, however, in order of apparent values and concentrations of preservatives.

Although delayed growth indicated more potency than is apparent from final results, the weakness of these substances still probably exceeds general belief, due to the fact that few have considered the question in the light of emergency use.

Broth is more likely to be negative than agar as here tested. These agar tests are, of course, not suited for regular sterility tests, but broth naturally furnishes the best conditions for bacteriostasis. Broth herein received 0.2 c.c. Agar was given only 0.1 c.c., but this was placed on the surface of a hardened plate, *i. e.*, full preservative strength. Quite possibly gradual diffusion of the preservative, leaving the living organisms, accounts for the results, a principle which might find use with further thought.

Serum has been known to spoil with this preservative. It cannot be classed as a good preservative, but there happens to be evidence that it has no appreciable effect on the virus neutralizing properties of poliomyelitis serum.

Merthiolate.—A fair germicide, but unusual in its high inhibiting effect at high dilutions. However, although broth transfers from preserved serum are likely to remain sterile, organisms may be demonstrated in serum preserved with 1:5,000 dilutions after hours or even days.

Glycerin.—Fifty per cent gylcerin finds many uses as a preservative, but a considerable number of organisms grow in media up to 5 per cent glycerin. Although 1 per cent glycerin in serum appears to inhibit somewhat, the action is probably slow for emergency use.

Formalin.—Although used extensively in vaccines, the use of formalin in serum has been avoided because of the effect on antibodies. It has fair preserving qualities, but data seem not to be available for more than a few antibodies, and these contraindicate its use.

COMMENT ON PROCEDURES USED

Tests which were performed locally were not as elaborate as might be desired; but the results lend themselves to logical conclusions, and it is believed that further refinements would be confirmative and would enlarge the scope rather than alter the conclusions. In brief, human serums were preserved with the stated concentrations of five products. To these staphylococci were added in concentrations of one drop of broth culture to 5 c.c. of serum, heavier, to be sure, than might be expected in practice. In order to cover practical conditions with a little more care, B. coli was used also as a test organism, not because it would be a usual contaminant, but because of the wide variation in the action of preservatives against different organisms. Serum was kept in the refrigerator, although some was tested at room temperature as a check of shipping and similar unavoidable clinical conditions.

The serum was tested by the inoculation of 0.1 c.c. to the surface of agar plates, spread with a glass rod, and the inoculation of 0.2 c.c. to 10 c.c. of broth. Attention might well be called at this point to the fact that a slight contamination, e. g., two organisms per c.c., would require the transfer of a fair quantity of serum to detect the contamination—and thus a large amount of preservative also. On the other hand, a heavy contamination, allowing a small transfer of preservative, does not represent working conditions. The proper, but impractical, procedure would thus be to make sterility tests in large flasks of media—representative portions of serum, and high dilutions of preservative. This, in condensed form, explains the difficulties of proper testing for sterility, and, incidentally, explains a large number of favorable tests of germicides which seem satisfactory, but are entirely fallacious. That the tests herein reported are not above criticism on this score is evident. But the use of plates as well as broth consistently demonstrated the presence of living organisms in serum which yielded negative broth tests. Also, the use of more organisms than might be considered practical contamination made possible the use of smaller portions of serum than would usually and properly be used. It must, however, still be stated that positive tests, or growth, under any conditions, condemn, whereas negative tests, or no growth, do not prove sterility.

RESULTS SUMMARIZED

The results may be briefly summarized as follows:

Tricresol.—Both broth and plate tests indicate survival of staphylococci for at least five days, the longest period tested, in serum preserved with 0.2 per cent tricresol. This product clouds the serum, and appears to act with some inhibition, but germicidal action is slow. The antiseptic properties seem sufficiently weak, so that interference with normal sterility tests should not be expected.

Chinosol.—Organisms survived not only in serum diluted 1:1,500 for at least six days, but also at 1:1,000, a recommended germicidal strength. Inhibition was definite, and delayed growth occurred in broth as tested. In short, brief sterility tests might easily yield false negative tubes, indicating sterile serum when it was actually contaminated.

Merthiolate.—At 1:5,000, the strength which has been used at Hooper Foundation, no growth was secured at and after forty-four hours of exposure of organisms to preserved serum kept in the refrigerator, although a few survivors were detected after six days in serum kept at room temperature. This may mean sterility, and certainly indicates some germicidal action as well as complete inhibition. Broth tests were negative, when plate tests were positive. With this substance, broth sterility tests will almost invariably be negative, regardless of whether serum is sterile. In 1:10,000 dilution inhibition was marked, but organisms survived for the longest period tested. Broth tests again were consistently misleading, and have undoubtedly been responsible for a false sense of security on numerous occasions. Although there were several delayed cultures, 1:50,000 merthiolate was at best a weak antiseptic. Apparently, for safe broth sterility tests the ratio of serum (1:5,000 merthiolate) to broth must exceed 1 c.c. of serum to 500 c.c. of broth.

Glycerin.—Although quantitative counts might well indicate some inhibiting effect, or preserving quality, cultures by the method used were consistently positive from tubes preserved with 1 per cent glycerin. Naturally, sterility tests would be accurate with this substance—below 1 per cent it is innocuous.

Formalin.—Formalin at 0.2 per cent not only preserved, but killed most of the staphylococci present in five days. It is a marked inhibiting agent, and broth sterility tests were at times misleading as used. The main objection to formalin thus is not its preserving properties, but the unknown action on the protective value of a serum.

IN CONCLUSION

Thus, of the substances tested, it would appear that merthiolate is the logical substance for emergency use, and even so too much must not be expected of it. Other substances should be used absolutely as preservatives, with complete and adequate sterility tests, not only for a time equal to federal requirements, but by methods which will give actual and not misleading results. Sterility tests with merthiolate are, for the most part, quite valueless. All such broth tests must be made with a high ratio of broth volume to serum, as high or higher than a ratio of 0.02 c.c. of 1:5,000 merthiolate preserved serum per 10 c.c. of broth, and it should be proven that broth with serum will support the growth of a very light inoculation of living organisms.

Third and Parnassus Avenues.

DISCUSSION

K. F. MEYER, PH. D. (University of California).—The timely report by Doctor Marshall deserves consideration in the light of recent experiences. In a circular letter sent to every physician, under date of June 1, 1934, the director of the State Department of Public Health, Dr. J. D. Dunshee, quoted my statement on the preparation and use of serum in the treatment and prophylaxis of poliomyelitis. "From the standpoint of preventive medicine, it commends itself on account of its simplicity and harmlessness, provided an organization can be perfected to secure an ample supply of properly tested serum. . . . *The collection and preparation of the convalescent sera should be conducted in a central station.*" To this should have been added: "In accordance with the federal standard specified for the production and testing of sera," since it is well known that in Europe a number of fatal accidents followed the use of contaminated sera employed in the prophylaxis against measles. Unfortunately, the advice to centralize the processing of human sera was not followed. Laboratories neither equipped nor staffed with personnel experienced in handling biologics rushed into the work of handling human blood. The consequences are known through the reports in the daily press. A two-year-old boy succumbed to staphylococcal poisoning, following the intramuscular injection of a specimen of human serum contaminated with cocci. The circumstances leading to the contamination are not clear. Since the risk exists, it is obviously advisable to use a germicide which will with certainty destroy the accidental contaminants. Such a preparation is merthiolate in a dilution of 1:5000. In order to prevent the recurrence of accidents similar to that reported from Healdsburg, it will in the future be necessary to delegate the processing of human sera and the handling of biologics to laboratories selected and approved by the State Department of Public Health.

PSITTACOSIS*

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DISCUSSION *by George H. Roth, M. D., Los Angeles*

UNDULANT fever and tularemia, when first recognized, were considered rather rare diseases carried to man by animals; but after more careful study it was found that these infections were widely distributed and by no means so uncommon, and had simply been confused with other acute illnesses.

The influenza epidemics, and various other waves of acute illness that affect the respiratory system, have probably acted as a screen to many psittacosis outbreaks, and have served to confuse the diagnosis so that this disease, too, has heretofore been considered a rarity, and has not received the study it deserves along with the other infections borne to man by animals or birds. It is by no means a new disease, as it has been recognized since the outbreak in Switzerland in 1879; and there have been many epidemics of varying proportions in the civilized world since that time, one of the most thoroughly studied outbreaks being that in Paris in 1891. From November, 1929, to May, 1930, the United States Public Health Service had records of seventy-four foci of infection giving rise to 169 cases with thirty-three deaths from psittacosis. These cases were distributed among fifteen states, and were traced to shipments of birds, belonging to the psitticine family, from widely separated tropical countries. At the same time, sporadic cases of the disease were found in practically all parts of the world. Prior to this time, however, it was practically unknown to our country; but since then, perhaps not a single month has passed without a report being made of the disease in the United States.

ETIOLOGY

This disease evidently originated in the parrot or psittacine family of birds, and hence its name. It would be a mistake, however, to believe that the parrot alone is responsible for the spread of the virus. He probably spread it in the first place; but by now a great many of the commercial aviaries, especially in California, are involved, and many of the cage birds, especially the budgerigars, are infected, and evidence points to the fact that practically all of these birds are capable of becoming carriers. The writer has seen two human cases definitely caused by canaries.

In 1892, Nocard,[1] in France, isolated a bacillus from the bone marrow of a dead parrot's wing and called it the Bacillus psittacosis, presumably suspecting that the parrot had died of psittacosis. Several investigators isolated this organism in dead parrots, and two Frenchmen, Gilbert and Fournier,[2] reported having found it in the blood of a patient at autopsy, and from the parrot which had been in this person's care. Others demonstrated that this organism belonged to the Salmonella group, which has a proven pathogenicity for animals, including man, being in the latter a common cause of food poisoning.

During the 1929-1930 epidemic, in the United States, an intensive search was carried on by Branham, McCoy and Armstrong,[3] of the National Institute of Health, for the Nocard bacillus, in the carcasses and droppings of parrots, and the material from human cases shipped them, but no strain of Bacillus psittacosis, or of any other member of the Salmonella group of bacteria was found. Their work has been verified by careful investigators, and the Nocard bacillus is no longer generally considered the causative organism.

McCoy[4] states that between January and March, 1930, eleven cases developed in the Hygienic

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